

Ribosomes: Development of Some Current Ideas¹

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INTRODUCTION

My purpose in this talk is to discuss how some present ideas of ribosome function developed. This involves a few anecdotes about the early days of ribosome research, about 10 years ago. Then ribosomes were just ceasing to be a laboratory curiosity. They had been observed as peaks in the ultracentrifuge (31), and pulse-labeling experiments had already shown that ribosomes are the sites of protein synthesis in living cells (2, 16). The particles had begun to be purified by Tissières and Watson (38), and it had become a feasible research project for a Ph.D candidate, like me, to get each size class of ribosome pure, measure the molecular weights, and do some other standard measurements. It even seemed feasible to try to study their function in extracts, for studies on amino acid incorporation had already shown that ribosomes in extracts are again the sites of protein synthesis.

THE TWONESS OF RIBOSOMES

With his typical intuition, Watson set to work on the ribosomes at that time with the flat statement that we would not understand protein synthesis until we understood the ribosome.

In my first discussion with him, Watson pointed out two of the most curious features of ribosomes (Fig. 1). One characteristic of all organisms is the twoness of their ribosomes. There are two ribosomes, usually characterized by their approximate sedimentation constants. The numbers that were assigned to them in the early work were 30S and 50S, and these became in a way the names of the two ribosomal subunits. The other characteristic was that not only were there two ribosomal subunits, but one of them is larger than the other. The fact that there are two components is curious. Why two particles, and why

one larger than the other? I took this problem as a personal challenge. The questions still cannot be answered, and my purpose in the rest of this summary is to outline the present state of our understanding.

While these ideas are often of general interest now, the ribosomal particles themselves at that time attracted little interest. The major source of excitement was the brilliant success of the famous adaptor hypothesis of Francis Crick (3). Appropriate transfer ribonucleic acid (RNA) molecules obediently brought each successive amino acid to the growing polypeptide chain. And the non-specific role of ribosomes in the process was a detail.

You may be interested in an account of one incident involved in the measurements of molecular weights of purified 30S and 50S particles. We already had other evidence that the molecular weights were probably in the ratio of 2:1, but, because of technical problems in keeping the 30S particles stable during purification, some of their proteins were often lost. The measured ratio of molecular weights therefore kept coming out rather higher than 2:1—more like 2.25:1. I was discouraged because it meant more work. But Watson cheered me up. He made the suggestion that “at last we can really interest Francis (that is Francis Crick, of course) in the ribosomes. We’ll send him a cable: ‘molecular weights of ribosomes now determined, ratio of 50S and 30S molecular weights is *e*.’ The base of natural logarithms (*e*) would have given the problem more appeal.

“ACTIVE RIBOSOMES”

With the separation and determination of the molecular weights of the ribosomes, we began to analyze some properties of protein synthesis by these ribosomes. Alfred Tissières and I then

¹ Eli Lilly Award Address (1969).

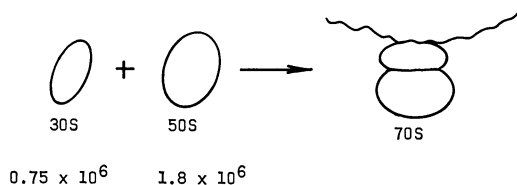


FIG. 1. Ribosomes of *Escherichia coli*. The ribosomal particles include the 30S and 50S subunits, with the approximate molecular weights indicated, which join together to form a 70S ribosome ("monosome") on messenger RNA.

found a property peculiar to those ribosomes that function in extracts (37). We already knew that the two ribosomal subunits—one 30S and one 50S particle—are joined together in a 70S particle when they form a protein chain. These 70S particles dissociated at low levels of magnesium ions, but the ones that had functioned in protein synthesis in extracts did not dissociate under conditions in which the bulk of the particles did. This is illustrated in Fig. 2, where we see a peak of the "active ribosomes," as we then named them.

The pattern is a standard one in a sucrose gradient of ribosomes from an extract of *Escherichia coli* prepared by grinding the cells with alumina powder. There is a peak in the profile which corresponds to the position of 70S particles. In this case, protein synthesis has been carried out in the extract, and much of the labeled protein remains bound to the ribosomes. When sedimentation analysis is carried out in a high Mg^{2+} concentration, much of the nascent protein appears bound to the 70S particles—these are the sites of the protein synthesis. However, a different result was observed when the extract was first dialyzed against low Mg^{2+} before the sedimentation analysis. Under these conditions, most of the 70S ribosomes dissociated to 30S and 50S particles (39). However, the fraction of ribosomes bearing the radioactive amino acids continued to sediment at 70S even at a concentration of magnesium at which the bulk of the ribosomes, which evidently had *not* functioned in the extract, had dissociated (37). Clearly, these experiments offered some hint about the twoness of the ribosome; that is, that somehow the ribosomal particles, a 30S and 50S particle joined together, were rather more stable to dissociation in low magnesium when they had functioned or were functioning in protein synthesis. However, this hint was far from enough to clear up the question.

Subsequent to this degree work, I was a fellow at the Pasteur Institute, where the scientific

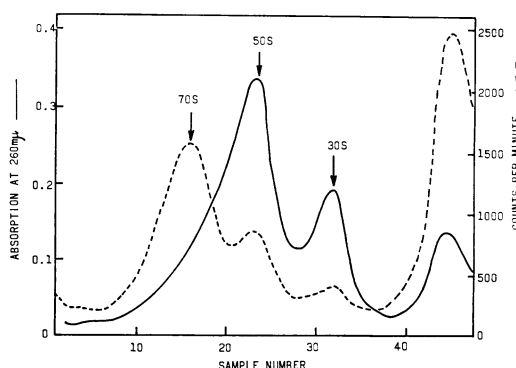


FIG. 2. Zonal sedimentation analysis of an *E. coli* extract after the incorporation of amino acids into protein was directed by endogenous messenger RNA. The reaction mixture was dialyzed against a buffer containing $10^{-3}M$ Mg^{2+} , and centrifuged through a sucrose gradient. Most of the ribosomes appear as 30S and 50S particles (38). A small number of 70S particles remain, bearing much of the labeled product [adapted from Tissières et al. (37)].

atmosphere was supercharged with excitement. Jacob and Monod were then writing their powerful review on messenger RNA (9), and their work on allostery was just coming to fruition. I reluctantly add that I had nothing to do with all this. With Francois Gros, Francoise Levinthal, and Dr. Monod, I started working on a then quixotic project, attempting to prove the messenger RNA hypothesis. The idea was to start with RNA isolated from a cell that had been actively synthesizing β -galactosidase, and use the extracted RNA to direct the synthesis of β -galactosidase in otherwise incompetent extracts. At that point in technology, this was over-optimistic, something like trying to pick up a needle with a steam shovel, and the results were negative. But in some side experiments, Francoise and I did find out one thing of interest about the so-called "active ribosomes" (33). We found that they contained pulse-labeled messenger RNA, as well as nascent protein, and they were not *irreversibly* jammed together. At even lower concentrations of magnesium ions, lower than those required for the inactive ribosomes to dissociate, the messenger RNA, 30S, and 50S particles of the active ribosomes could also dissociate, and could then be detected moving independently of one another in sucrose gradients (33). The problem now became how ribosomes joined to messenger RNA and were released from it, and the relation of the 30S and 50S ribosomes to messenger RNA. What stabilized the active ribosomes against dissociation?

POLYRIBOSOMES

In fact, when I returned to the States at that time, and joined Washington University in St. Louis, the problem of analysis of ribosome function had greatly changed. Along with the notion of messenger RNA had come the idea of the polyribosome—a single messenger RNA servicing a number of ribosomes at the same time. This notion of the polyribosome is represented in a familiar way in Fig. 3 (40, 41). A messenger RNA molecule is depicted with a number of ribosomes attached to it. Each 70S ribosome, containing one 30S and one 50S subunit, travels along the messenger RNA and forms a polypeptide chain as it moves in response to the information encoded in the messenger. As soon as the ribosome has moved far enough along the messenger to have translated a section of it far from the initiation point, another ribosome can add at the initiation point and begin to move in tandem behind the first. Thus, an efficient system can be generated in which a single messenger RNA can function with a number of ribosomes at the same time. This notion was thoroughly worked out by Gilbert in Watson's lab (6), and then Marks, Burka, and I (22)—and independently, Gierer (5) and other workers—found evidence for bulk quantities of polyribosomes in rabbit reticulocytes. Later Schaechter (32) and I (34) found ways to detect large quantities of polyribosomes in lysates of bacteria.

It was by then clear that many of the ribosomes originally observed as 70S particles in cell extracts were actually fragments of polyribosomes. The very large polyribosomal structures were intrinsically highly unstable. They were unstable in the cells, where component messenger RNA only lasted minutes; and they were unstable in extracts, where shearing forces and nuclease action rapidly split them at weak points into small polyribosomes and 70S particles. Because it took so long to harvest bacteria, and techniques like alumina grinding and sonic treatment that we used to open the cells were so harsh, it seemed probable that the distribution of ribosomes changed greatly during the preparation of the cell extracts.

One way to try to analyze the process by which polyribosomes truly form was to work in extracts, starting with ribosomes and messenger RNA. But no system was then, or is even now, available to study controlled formation of polyribosomes in extracts. There were also indications that fragmented polyribosomes and 70S particles could start to function on new messenger RNA by a process in which they left one messenger RNA and moved to another before finishing a

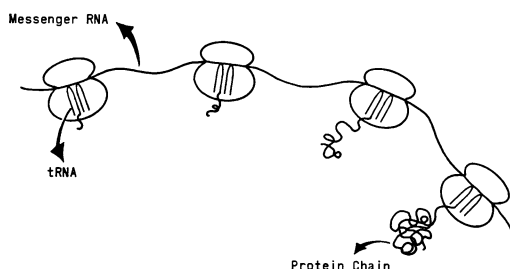


FIG. 3. Polyribosome, showing four ribosomes moving in tandem along a molecule of messenger RNA. Each carries peptidyl transfer RNA and another transfer RNA bringing in the next amino acid to be polymerized. The protein chain grows progressively in length as the ribosome moves toward the end of the translated sequence of messenger RNA [adapted from Watson (40)].

protein chain (27); such a process is certainly not identical to that in whole cells, where one hardly expects ribosomes to leave one messenger RNA and join another in mid-course. Furthermore, there are some questions—like the precise kinetics of messenger RNA breakdown, the rate of RNA synthesis, and a variety of others—that *must* be investigated in whole cells.

POLYRIBOSOMES FROM FRAGILE CELLS

We therefore set ourselves the problem of finding a way to release all the ribosomes from actively growing cultures essentially instantaneously, and with as little disturbance of the *in vivo* distribution as possible. This project became feasible because of the fortunate arrival of two collaborators. One, David Apirion, is a geneticist, with whom a most enjoyable collaboration has continued. Many biologists agree that geneticists think in a more original way than the rest of us; and judging by one case, Israeli geneticists are among the most original. Apirion suggested that, since ordinary cells are so difficult to open, one might instead use mutants, mutants that would grow exponentially, but in a highly fragile form, and which therefore could be lysed by mild procedures. We managed this by selecting sucrose-dependent strains of *E. coli*. These strains can be grown in an osmotically sensitive form, and are protected by sucrose added to the medium (17). Many such strains can be easily lysed, like penicillin spheroplasts, but, unlike spheroplasts, they grow exponentially. Another technique was also developed, a physiological one, in which many strains of *E. coli* could be grown in fragile form in the presence of high salt concentrations (19).

As is well known from work with sphero-

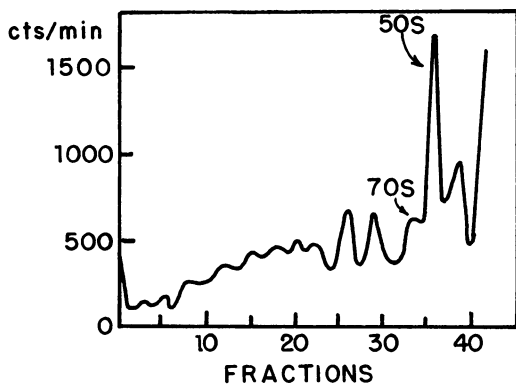


FIG. 4. Ribosome distribution in zonal sedimentation analysis of a lysate of fragile cells. The ribosomes are detected by their content of ribosomal RNA labeled with ^{14}C -uracil. From right to left are displayed 30S and 50S ribosomal subunits, 70S monosomes, and polyribosomes containing 2, 3 . . . n monosomes [adapted from Mangiarotti and Schlessinger (21)].

plasts and L-forms, it is very difficult to separate growth of bacteria from intactness of the cell envelope. To get conditions in which reproducible cultures could be grown in fragile form was hard work, and was possible only through the resourcefulness of Giorgio Mangiarotti, who was with us from 1965 to 1967. After hundreds of growth curves and about 900 sucrose gradients, Mangiarotti came up with a characteristic sucrose gradient pattern of ribosomes in extracts (20). The pattern is the result of attempts to minimize nuclease action, shear, and polypeptide chain completion during the course of preparation of the lysates. This characteristic pattern is shown in Fig. 4.

There are three features of this sucrose gradient that were different from the patterns previously obtained in our laboratory and in other laboratories. First of all, the pattern showed a rather flat distribution of polyribosomes, as opposed to the peaked distributions seen earlier, and it also showed polyribosomes much more fast-moving and presumably much larger than the ones that had earlier been seen. We attributed these differences from the earlier patterns to the minimization of breakdown. Instead, in the earlier patterns, many of the large polyribosomes had been broken down to smaller ones and even to 70S particles.

The second feature of the gradient, unexpected at that time, was the very small number of 70S particles. Instead, a sizable number of free—or, as they are sometimes called, “native” (7)—30S and 50S subunits were observed.

The third unusual feature of the gradients

only appeared when one began to determine the localization of different kinds of RNA. It was then found that, if one looked at newly formed RNA (by labeling it with tritiated uracil, for example), one found that all the messenger RNA of growing bacteria is found in polyribosomes. In other words, in Fig. 4 one can draw an imaginary vertical line between the 50S particles on the right, and the 70S particles and polyribosomes on the left. To the left of the line was found all of the messenger RNA of bacteria.

If one then carried out a labeling experiment in which radioactive uracil was followed into cellular RNA, the radioactive messenger RNA would appear in the polyribosomes. As the unstable messenger RNA broke down and was replaced by new messenger RNA, the specific content of radioactive messenger RNA in the polyribosomes would increase until all of the messenger RNA was labeled. At that point, the content of label in polyribosomes in the form of messenger RNA would become constant, and would thereafter increase only slowly, in proportion to net growth of the cells. In short, by looking at the flow of new messenger RNA into the polyribosomes and the rate at which it saturated with label, an estimate could be obtained of the time it took to breakdown and replace all the messenger RNA that existed at a certain time (21). In earlier experiments, this kind of analysis had not been possible, because, with the breakdown of the polyribosomes, fragments of messenger RNA could be found floating in various parts of the gradient—sometimes free from ribosomes and contaminating other fractions, sometimes completely degraded and lost (25).

In the right-hand part of the gradient, short of the polyribosomes in the sucrose gradient analysis, one found that all the newly labeled ribosomal RNA (the stable RNA in the cells) continued to sediment for some time after its formation.

In spite of the fact that the chains only take several minutes to form, it takes 6 or 7 min before any of the ribosomal RNA enters polyribosomes in these cultures. The reason for this is that, in growing cells, the formation of ribosomes is not instantaneous. It takes about 1 min to make the RNA chain, but it takes much longer than that to coat it with the various ribosomal proteins required to transform the RNA chain into a complete ribosome. It is only when complete ribosomes have been formed that one can detect the newly formed ribosomal RNA moving into polyribosomes in the form of the complete subunits (18).

With detailed labeling experiments and DNA/RNA hybridization, the two regions of the sucrose

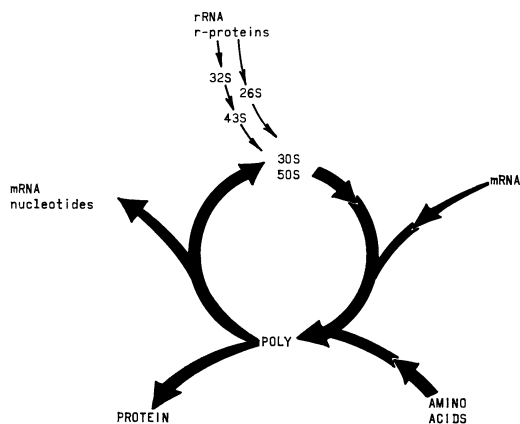


FIG. 5. Ribosome cycle in protein synthesis. Ribosomal subunits are formed from ribosomal RNA and proteins through respective precursors. One 30S and one 50S particle periodically join to a chain of messenger RNA as it forms and cycle across it as a monosome forming a protein chain as it moves. At the end of the messenger RNA sequence, the ribosomal subunits leave the messenger RNA and dissociate once more to rejoin the free pool.

gradients that are delineated—the polyribosomal region and the portion of gradient free from polyribosomes—were explored in more detail. We were able to work out a number of details of the way in which ribosomes add to messenger RNA and come off messenger RNA in intact cells.

The total analysis that we now believe to hold overall is shown in Fig. 5. In the cultures of *E. coli* that we used, it takes 1 to 2 min to form a chain of ribosomal RNA. These cultures are growing slowly, with a doubling time of about 2 hr. If the cells are growing faster, all of the events described are increased in rate. The ribosomal RNA then spends approximately 10 min more, on the average, being coated with proteins and moving through a series of precursors. There is one detectable holdup point at which an incomplete particle stops for some time on its way to becoming a 30S ribosome. There are two detectable holdup points (two precursors) that are detectable before the nascent 50S ribosome reaches its final form (18).

The messenger RNA also takes about 1 to 2 min to form, and ribosomes attach to it as it forms, so that by the time a messenger RNA molecule is finished it is in the form of a complete polyribosome (21).

THE RIBOSOME CYCLE

As the ribosomal subunits are finished, the old and the new ones periodically join to a chain

of messenger RNA, move across it in the polyribosome forming a polypeptide chain, and then are released at the other end. One can thus set up a primitive kind of ribosome cycle, as shown in Fig. 5.

However, to return to a point made earlier, there was another feature of the initial sucrose gradients that caused some interest and made the cycle more specific. There was a fraction of ribosomes in the lysates in the form of free 30S and 50S particles. These particles carried no detectable messenger RNA. They were not in polyribosomes and it was curious that they did not tend to associate together to form 70S particles at high magnesium concentrations (at least not to any extent measurable in the gradients).

The notion immediately suggested itself that perhaps these free particles were not, as some earlier workers had thought, incomplete ribosomes of some kind, or damaged ribosomes that were no longer functioning in the cells, but instead formed a true intermediate in the functional life cycle of the ribosomes.

Once again, we were thinking back to the curious “active ribosomes,” which were stable to dissociation in low Mg^{2+} . Now we extended the thinking to realize that not only were functioning ribosomes stable to dissociation, but they tended to come apart spontaneously when they were not functioning. That is, the protein synthesis *itself* is responsible for putting together the 30S and 50S partners as well as stabilizing them in the “active” complex.

The notion suggested at the time was that the 30S and 50S particles come together in a stable way to form a 70S unit only when the 70S unit begins to move across the messenger RNA (20, 21, 35). At the other end of the messenger RNA, when the protein synthesis is finished, the 30S and 50S partners are much less strongly associated and tend to dissociate, so that the 30S and 50S particles change partners after passing across messenger RNA.

We hesitated at first to submit this suggestion for publication, because of auxiliary data. In principle, the most direct method to discover whether the 30S and 50S ribosomal particles exchange partners during growth would be to use the technique of equilibrium centrifugation in CsCl gradients. The experiment would be analogous to the DNA transfer experiment carried out by Meselson and Stahl (24). A number of workers had tried to do such experiments (23). However, the attempts had been frustrated by instability of ribosomes in CsCl. Furthermore, one of the most prestigious laboratories in the field had privately circulated some conclusions

from partially successful experiments, suggesting that 30S and 50S ribosomes did not change partners very often during growth, but that individual 70S ribosomes seemed to persist as such for some time in growing cells. Furthermore, everyone had grown accustomed to seeing and thinking of "stable 70S ribosomes." They had been my own meal ticket for almost a decade, and it was with great reluctance that I modified my own ideas.

Nevertheless, our labeling studies (21) forced us to conclude that the cycle we had imagined probably was right. Soon afterward, a version of the CsCl transfer experiment was successfully completed by Kaempfer, Meselson, and Raskas (10), and proved that ribosomes often do change partners during growth. By now, the cycle has received a good deal of comment and is supported by many experiments from a number of laboratories. Many details have been worked out, and it is widely believed to hold pretty much in the simple form that was first suggested. [For the present state of thinking and information on the details of the ribosome cycle, see our forthcoming review and related articles (Cold Spring Harbor Symp. Quant. Biol., *in press*).] For example, the 30S and 50S particles found free in extracts will come together and can indeed function in protein synthesis; but in order to join them together, one must provide magnesium and potassium ions, messenger RNA, and transfer RNA, in fact, the ingredients required to put together a complex that can initiate protein synthesis (11, 35). The notion is thereby supported that the free particles are temporarily out of a job, free from messenger RNA but capable of rejoining it in the presence of the appropriate conditions to start protein synthesis again.

ANTIBIOTIC BLOCKS OF THE RIBOSOME CYCLE

If the notions of a ribosome cycle are of importance, then it should have some predictive value in analyzing various circumstances in which the activity of the ribosomes is affected; in particular, one can ask about instances in which the cycle of ribosome function is blocked. The approach here is very comparable to that when one suspects the existence of any biochemical cycle. A true cycle should have inhibitors that block it in characteristic ways, so that intermediates proximal to the block pile up. Three major ways to stop ribosome function have been employed. The first way is nutritional: for example, cells that require a particular amino acid for growth can be starved of the amino acid, and of course, protein synthesis will then stop, or

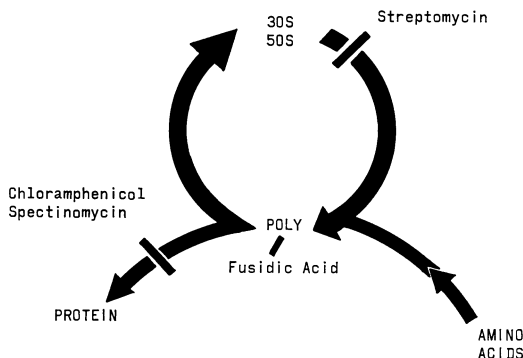


FIG. 6. Blockage of the ribosome cycle by some antibiotics.

alternatively, the cells can be starved for glucose and, when the energy supply is lowered sufficiently, protein synthesis will tend to stop.

Another method that can be used for blocking the ribosome cycle in some way is mutational; that is, mutants can be isolated that at least under some conditions cease to form protein, and it can be asked what happens to the ribosome cycle in these cases. The arrest of the ribosome cycle in these cases can be more or less characteristic; it can be a secondary consequence of other events, or it can have a fairly specific character.

However, the most direct method of blocking the ribosome cycle, and an especially interesting one, I think, for microbiologists, has been the use of specific antibiotics that are known to stop ribosome function. Of a number that have been investigated in the laboratory, these tend to fall so far into three groups, which are indicated in Fig. 6. The first group includes streptomycin and certain other aminoglycoside antibiotics such as neomycin (13-15). The second group includes a variety of antibiotics like chloramphenicol, erythromycin, and spectinomycin (8, 8a). The third group includes fusidic acid (8), and probably other antibiotics as well (4).

In the case of streptomycin and neomycin, there is a characteristic arrest of the ribosome cycle, illustrated in Fig. 7. At the left is a sucrose gradient analysis of a lysate made just prior to the time of addition of the drug; at the right is a comparable one of the distribution of ribosomes 40 min after addition of streptomycin. The obvious effect of the drug is to cause an enormous rush of polyribosomes and many 30S and 50S particles into material sedimenting at 70S.

When one saw such a huge accumulation of 70S particles, the natural question was: what are they? According to the simple ribosome cycle we have been describing, such 70S ribo-

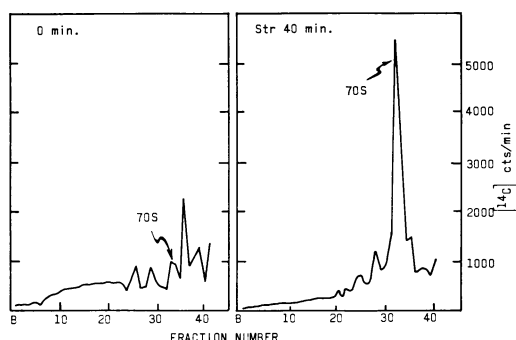


FIG. 7. Sucrose gradient zonal sedimentation of a lysate of growing fragile cells made at the time of addition of 75 μ g of streptomycin per ml (left) or 40 min later (right).

somes could arise in one of two ways. First, ribosomes could reach the end of messenger RNA and leave it, but not dissociate. Such a 70S ribosome would thus result from a block at termination. Alternatively, ribosomes could finish protein chains in the presence of the drug, and dissociate to 30S and 50S particles. However, when the 30S and 50S subunits rejoined on a new messenger RNA, the function of the new complex could be blocked by streptomycin. Such a 70S ribosome would result from a block at initiation.

These alternatives can be differentiated in a simple way: if blockage is at termination, the 70S ribosomes will have been released from messenger RNA. On the other hand, if blockage is at initiation, each 70S ribosome will bear messenger RNA. With Lucio Luzzatto, we then looked and found that messenger RNA was present in the accumulated 70S ribosomes, so that the most severe blockage seems to be at initiation (14).

Our present model is something like this. The 30S and 50S particles that are already functioning in 70S form continue to move across messenger RNA, at least slowly, in the presence of the drug, reach the end of the messenger RNA sequence that is being translated, and dissociate. When they reassociate at the beginning of messenger RNA chains, some have not been hit by streptomycin, and can cycle once more. But many ribosomes bind streptomycin during the initiation process, and those particles are blocked at that point. In other words, the initiation complex containing streptomycin and 30S and 50S particles forms, but moves little if at all. The result of this action of streptomycin is that there accumulate in the cells large numbers of particles that move in lysates at 70S, each containing a 30S and a 50S particle, and, so far as one can tell, transfer RNA and a complete chain of

messenger RNA. The accumulation of these blocked particles is irreversible, and can account for the lethality of streptomycin.

The action of chloramphenicol and the other antibiotics of the second group is unexpected. It is dominated by the peculiar feature that the ribosome cycle appears to continue, even though little or no peptide bond formation (i.e., little or no protein synthesis) is going on (8a). In these cases, there may be a true uncoupling of two processes: the process of ribosome addition and movement along messenger RNA, which continues in the presence of the drugs; and the process of peptide bond formation, which is arrested in the presence of these drugs. That is, translation in the sense of moving ribosomes along the messenger RNA would continue, but polypeptide formation would be blocked.

The case of fusidic acid is quite an interesting one because its specific function is known (30, 36). From work in the laboratory of Tanaka (36), it is known that fusidic acid blocks the activity of one of the factors of protein synthesis, the G factor, an enzyme which is responsible for the hydrolysis of guanosine triphosphate and which Conway (2) and Nishizuka and Lipmann (28) had first suggested to be involved in moving ribosomes along messenger RNA. One might then expect that in the presence of this drug, ribosome movement on messenger RNA would stop and the polyribosomes would freeze as such. Generally, that is what one finds (8). In the cultures treated with fusidic acid, messenger RNA continues to add to polyribosomes but at a much slower rate than in the control, and primarily in the smallest polyribosomes. Polyribosomes present at the time of addition of the drug remain extractable from the cells even 1 hr or more after the time when the drug has been added.

PROSPECTS

At this point, the cycle of ribosome function seems to be well established; it has helped to account for the actions of certain antibiotics, and, in turn, the actions of the antibiotics have shown that, with the production of characteristic lesions, one can demonstrate certain detailed features of the ribosome cycle.

The results continue to have a number of implications for other questions of interest about ribosomes. For example, there are implications in the discussion of the control of the lifetime of messenger RNA, and in the movement of ribosomes along messenger RNA.

We do not know at present how messenger RNA is degraded in whole cells, nor do we know what determines how long it will last before a

chain of messenger RNA begins to be degraded. It is of interest, therefore, that in the presence of many of the antibiotics that we have looked at, messenger RNA breakdown tends to stop; that is, the messenger RNA bound to ribosomes in the presence of these antibiotics is stabilized against degradation (8a). The reason for this is unclear, but it is clearly a hint to the mechanism by which the messenger RNA lifetime is ordinarily controlled.

There are comparable hints that relate to the genetics of the ribosome and to the mechanism of movement of the ribosome along messenger RNA. For example, the results just mentioned, in which fusidic acid does block entry of messenger RNA into polyribosomes, argue strongly that *in vivo* guanosine triphosphate hydrolysis by G factor is truly a critical feature of translation of ribosomes along messenger RNA. In contrast, the results with chloramphenicol argue that protein synthesis *per se* (that is, bond formation) is not required for the movement of ribosomes along messenger RNA. How these results can be reconciled with the common notion held before, that ribosomes move along messenger RNA in keeping with polypeptide formation, is not at all clear at this point. Most of the discussion is speculative, and need not detain us further now.

I can't close, however, without recalling the obstinate refrain of the question of the size and twoness of the ribosomes. Unfortunately, all of the results, while they continue to suggest some features of ribosome function and cell physiology, still do not tell us why the ribosome has two subunits or why one is twice the size of the other. Our picture of the "active ribosome" is now much more elaborate, and we now know that in fact the two ribosomal subunits—at least in bacteria—tend to separate at the end of the formation of a protein chain and exchange partners before they begin another, but this is not an argument that this process *must* occur; nor is it an argument which explains *why* it does occur. As often happens in science, we begin by asking why, and end by finding out what.

The true reason for the twoness and size of the ribosomes is likely hidden in the details of function of the ribosome on messenger RNA. The messenger and anticodon of transfer RNA bind to the 30S particle, while the other end of the transfer RNA is bound to the 50S ribosome. The two particles must join together, but not too tightly, to permit the appropriate relative movement of all the intricate parts of the polyribosome.

It's not clear to me whether that last statement represents much more than words, and I am therefore ending my talk with a frank admission

of failure in my original purpose, but I hope that in this way I can convey to you that the work is still very much in progress, and share with you some of the excitement and anticipation of the future that all of us working on ribosomes now feel.

Author's Note

With the option of publishing the Lilly Award Address as it was presented or expanding it into a review, I have chosen to let the talk stand.

This has two serious drawbacks. First, the talk of course is brief and emphasizes work in which I have participated myself, so that other groups of workers may well feel slighted. Second, as the text indicates, the recent work described was part of a collaborative effort with David Apirion, and most of the development of the experimental system was done by Giorgio Mangiarotti. The presentation of an award to one person tends to obscure that.

With regard to the first point, a number of reviews by others (4a, 12, 29, 42), and two by Apirion and me (32a; Cold Spring Harbor Symp. Quant. Biol., *in press*), already cover relevant material in depth, and can counteract my biases with more balanced views. With regard to the second deficiency, I can only hope that my statements will be taken as matters of fact.

ACKNOWLEDGMENTS

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ADDENDUM IN PROOF

Additional work of Michihiko Kuwano and Cathy Kwan has demonstrated a potent new ribonuclease activity ("RNase V") which is dependent on the translocation of ribosomes on messenger RNA (M. Kuwano, C. Kwan, D. Apirion, and D. Schlessinger, Proc. Nat. Acad. Sci. U.S.A., 1969, *in press*; First Lepetit Symposium on RNA Polymerase and Transcription, 1969, *in press*). Like the ribosome-dependent guanosine triphosphatase (2, 28), it requires the formation of monosomes from ribosomal subunits. Thus, the mechanism of periodic separation and coupling of ribosomal subunits may have been evolved to help prevent needless guanosine triphosphate hydrolysis and inactivation of messenger RNA.

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